

CLAIMS

1. A method of screening enzymes for variants with improved specific activity, comprising the steps of

- 5 (i) generating a library of nucleic acid sequences encoding enzyme variants of interest
- (ii) providing a nucleic acid sequence encoding an enzyme to be fused with the enzyme in (i)
- 10 (iii) fusing nucleic acid sequence encoding enzyme variants in (i) with nucleic acid sequence encoding enzyme in (ii)
- (iv) transforming the fused nucleic acid sequence obtained in (iii) into a host cell
- (v) culturing host cell in (iv) in order to express the fused enzymes
- (vi) sampling each cell culture obtained in (v)
- 15 (vii) analyzing samples obtained in (vi) by determining activity ratio of the expressed fused enzymes
- (viii) selecting the samples exhibiting the desired activity ratio.
2. The method according to claim 1, where the enzymes are fused by means of a linker by fusing nucleic acid sequence encoding enzyme variants in 1(i) with nucleic acid
20 sequence encoding a linker and further with nucleic acid sequence encoding enzyme in 1(ii).
3. The method according to claim 2, where the linker consists of 1-40, or 2-20, or 2-10 amino acids.
- 25 4. The method according to claim 2, where the linker is selected from the group consisting of Poly-Arg, Poly-His, PEPTPEPT, FLAG, Strep-tag II, c-myc, S-, HAT-, 3xFLAG, Calmoludin-binding peptide, Cellulose-binding domain, SBP, Chitin-binding domain, Glutathione S-transferase, Maltose-binding domain.
- 30 5. The method according to claim 1, where the library is generated by mutating a nucleic acid sequence encoding a wild type enzyme.
- 35 6. The method according to claim 1, where the library is generated by mutating a nucleic acid sequence encoding a protein engineered enzyme.
7. The method according to claim 1, where the enzyme variant in 1(i) is generated by genetic engineering.

8. The method according to claims 5-7, where the enzyme is selected from the group consisting of proteases, cellulases (endoglucanases), β -glucanases, hemicellulases, lipases, peroxidases, laccases, α -amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, pectin lyases, mannanases, pectin methylesterases, cello-biohydrolases, transglutaminases and phytases.
9. The method according to claim 1, where the enzyme in 1(ii) is selected from the group consisting of proteases, cellulases (endoglucanases), β -glucanases, hemicellulases, lipases, peroxidases, laccases, α -amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, pectin lyases, mannanases, pectin methylesterases, cello-biohydrolases, transglutaminases and phytases.
10. The method according to claim 1, where the host cells in 1(iv) are selected from bacterial cells.
11. The method according to claim 10, where the host cells belong to a strain selected from the group consisting of the species *Bacillus alkalophilus*, *Bacillus agaradhaerens*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus clausii*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Streptomyces lividans* and *Streptomyces murinus*.
12. The method according to claim 1, where the host cells in 1(iv) are selected from fungal cells.
13. The method according to claim 12, where the host cells belong to a strain selected from the group consisting of the genera *Acremonium*, *Aspergillus*, *Fusarium*, *Humicola*, *Myceliophthora*, *Neurospora*, *Penicillium*, *Thielavia*, *Tolypocladium*, *Trichoderma*, *Eupenicillium*, *Emericella*, *Eurotium*, *Allomyces*, *Blastocladiella*, *Coelomomyces*, *Achlya*, *Candida*, *Alternaria*, *Rhizopus* and *Mucor*; preferably the species *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus niger*, *Aspergillus nidulans* or *Aspergillus oryzae*.

14. The method according to claim 1, where the host cells in 1(iv) are selected from yeast cells.

5 15. The method according to claim 14, where the host cells belong to a strain selected from the group consisting of the genera *Candida*, *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Candida*, *Pichia*, *Hansenula*, or *Yarrowia*, preferably to the species *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces*
10 *norbensis*, *Saccharomyces oviformis*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Hansenula polymorpha*, *Pichia pastoris*, *Yarrowia lipolytica*, *Schizosaccharomyces pombe*, *Ustilago maylis*, *Candida maltosa*, *Pichia guilliermondii* and *Pichia methanolicus*.

15 16. The method according to claim 1, where the fused enzymes in 1(v) is an extracellular product.